Allergic sensitization without detectable specific serum IgE

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July 27, 2022

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"To the Editor"

In clinical practice it is sometimes observed that allergic patients exhibit allergic symptoms or positive skin prick test results in the absence of detectable allergen-specific IgE in serum. In this study, twenty five subjects, age from 13 to 59 years [Mean 32.6±13.6], 16 males and 9 females, with birch pollen-related allergic rhinoconjunctivitis (ARC) with or without allergic asthma (AA), with (group 1: n=13) or without cross-reactive food allergy to apple (group 2: n=12) (oral allergy syndrome, OAS) were enrolled (Table E1) and investigated at three time points (i.e., before the birch pollen season-time point 1, shortly after the birch pollen season-time point 2 and thereafter in autumn-time point 3) (Figure 1). The diagnosis of birch pollen allergy and OAS, demographic, serological and clinical features of the patients are described in the Supplemental materials (Table E1). Two patients from group 1, (patients 1 and 2) with apple-related OAS stood out. They contained low but clearly positive Bet v 1-specific IgE levels before the birch pollen season (time point 1, patient 1: 0.16 kUA/L; patient 2: 0.11 kUA/L), showed increases of Bet v 1-specific IgE shortly after the birch pollen season (time point 2, patient 1: 0.75 kUA/L, patient 2: 0.53kUA/L) which then declined again until September at time point 3 (patient 1: 0.53 kUA/L; patient 2: 0.43 kUA/L) (Table E2, Table E3, Figures 2 and 3). Interestingly, Mal d 1-specific IgE could not be detected in the sera obtained from patients 1 and 2 at any of the three time points even by sensitive and quantitative ImmunoCAP technology (Table E2; Figure 2 and 3).

The two patients were young females who suffered from birch pollen-related allergic rhinoconjunctivitis and one (patient 1) suffered also from birch pollen-induced asthma (Table E4). Importantly, both patients suffered from OAS upon consumption of apples, especially after the birch pollen season and were positive in open food challenge with apple (Table E4). The two patients were sensitized only to few other allergens besides Bet v 1 and Mal d 1 and sensitization to other cross-reactive food allergens (e.g., profilin, lipid transfer proteins could be excluded (Table E5).

An increase of Bet v 1-specific IgE levels was observed in both patients 1 and 2 at time point 2 shortly after the birch pollen season (Figure 2 and 3, Table E2) which was associated with a strong increase in Bet v 1-specific basophil sensitivity in patient 2 (Figure 3, Table E6). The Bet v 1-specific basophil sensitivity in patient 1 at time points 1 and 2 was comparable whereas at time point 3 patient 1 showed basophil activation with a tenfold lower Bet v 1 concentration indicating increased Bet v 1-specific basophil sensitivity at time point 3 (Figure 2, Table E6). Exposure of basophils to anti-IgE antibodies indicated strongly reduced IgE-mediated basophil sensitivity in patient 1 at time point 3 as compared to time points 1 and 2 (Table E6).

We could not detect at any of the three time points Mal d 1-specific IgE in the sera of patients 1 and 2 (Table E2, Figures 2 and 3) but we found a strongly increased Mal d 1-specific basophil sensitivity in both patients at time points 2 and 3 (Figures 2 and 3, Table E6) after the birch pollen season indicating increases of Mal d 1-specific IgE on the patients basophils after birch pollen exposure. The increase of Mal d 1-specific basophil sensitivity was associated with marked increased apple-induced skin responses at time points 2 and 3 after the birch pollen season as compared to time point 1 before the birch pollen season (Figures 2 and 3, Table E7). By contrast, skin responses to histamine were comparable at all three time points in patients 1 and 2 (Table E7).

Using purified allergen molecules, our study has demonstrated that basophils and mast cells of allergic patients contain already allergen-specific IgE before it can be detected in serum in the form of free-allergen-specific antibodies. This finding is important because it demonstrates that allergen-specific IgE sensitization may already occur before allergen-specific IgE can be detected as free IgE in serum (Figure E1). This indicates that the early detection of allergen-specific IgE sensitization may require basophil activation, skin testing and/or provocation testing with defined allergen molecules to detect IgE bound to cellular IgE receptors before it can be traced in serum (Figure E1). Our conclusions are supported by an earlier study which has demonstrated that after administration of omalizumab, a monoclonal anti-IgE antibody which prevents allergen-specific IgE from binding to the high and low affinity IgE receptor, IgE sensitizations to allergen sources became detectable in serum which were undetectable before administration of omalizumab [1]. This effect would be explained by the fact that omalizumab blocked the binding of low levels of IgE specific for the allergen sources to cellular IgE receptors so that they became measurable in serum which is in agreement with the notion that application of omalizumab generally increases levels of allergen-specific free IgE in serum.

Our results are important for diagnosis of IgE-mediated allergic sensitization in general because they reveal that a certain threshold of allergen-specific IgE levels must be reached which exceeds the capacity of cellular IgE receptors in the body to bind IgE before it can become detectable as free IgE in serum. This must be taken into consideration when patients suffer from allergic symptoms but IgE specific for the corresponding allergens cannot be detected. In such cases basophil activation or skin testing with defined allergen molecules should be considered.

Our findings are also important when it comes to the investigation of the inception (i.e., time window of sensitization) of allergic sensitization in children because they indicate that early allergen-specific IgE sensitization may not be accurately detected by serology. This may apply for subjects with low allergen-specific IgE levels who are sensitized only to few allergen molecules. In fact, monitoring of allergen-specific IgE to multiple allergen molecules in birth cohorts indeed showed that children are often sensitized only to few dominant allergen molecules [2, 3, 4]. This may have important consequences for strategies for allergen-specific prevention because it may affect the definition of primary versus secondary prevention depending on the accurate definition at what time point in life allergic sensitization has indeed occurred. Finally, our data may explain why certain patients show allergic symptoms and/or allergen-specific positive challenge test (e.g., skin test) and basophil activation results although no specific IgE can be detected in serum. It is a limitation of our study that we have investigated only 25 birch pollen allergic patients but among those approximately 10% of patients showed a discrepancy between detection of specific IgE by tests based on effector cell activation versus IgE serology. Larger studies will be needed to determine the percentages of patients who present only effector cell bound specific IgE without detectable specific IgE in serum. Since we are currently running out regarding high quality allergen preparations for skin testing [5] such studies will need to be performed with purified allergen molecules performed under Good Manufacture Practice (GMP) conditions suitable for clinical trials but they are not available at the moment.

The strength of our study is that it was conducted with highly purified allergen molecules and hence delivered unambiguous results because they demonstrate the presence of allergen-specific IgE on basophils and in the tissues of allergic patients without detectable allergen-specific IgE in serum.

KEYWORDS: Basophil activation test, allergen-specific IgE, basophils, birch pollen allergy, cross-reactive

food allergy, Bet v 1, Mal d 1, oral allergy syndrome.

WORD COUNT : 1222 words, 3 figures.

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Author Contributions: RV, AOL, AAN, MRK, DAK, OGE designed the research studies. AOL, SEV, OGE, ESF, NII performed clinical work. AOL, MGB, SEV, AAN, OA, RC performed experiments. RV, AOL, MGB, SEV analyzed and interpreted the data. AOL wrote the manuscript with contributions from RV and SEV.

Acknowledgements: This study was supported by a Megagrant of the Government of the Russian Federation, grant number 14.W03.31.0024, by the Danube ARC grant of the country of Lower Austria, by the FWF-funded project P34472-B from the Austrian Science Foundation (FWF), and by the RUDN University Strategic Academic Leadership Program.

Disclosures and potential conflicts of interest: Rudolf Valenta has received research grants from Viravaxx AG, Vienna, Austria, HVD Biotech, Vienna, Austria and Worg Pharmaceuticals, Hangzhou, China. He serves as a consultant for Viravaxx and Worg. The other authors have not conflicts of interest to declare.

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FIGURE LEGENDS

FIGURE 1. Schematic representation of experimental schedule. Flags P1 (point 1) and P2 (point 2) represent blood collection dates for patients before (period 1) and shortly after the birch pollen season (period 2) and in September (period 3). The birch pollen season was determined according to pollen monitoring data.

FIGURE 2. A, Bet v 1-specific IgE levels (y-axis: kUA/L), B, Bet v 1-specific basophil activation (y-axis: percentages of activated basophils) in response to three different concentrations of Bet v 1 (x-axis) C, Mal d 1-specific IgE levels (y-axis: kUA/L), D, Mal d 1-specific basophil activation (y-axis: percentages of activated basophils) in response to three different concentrations of Mal d 1 (x-axis), and E, skin sensitivity to buffer (negative control), histamine (positive control) and apple determined at three different time points (Figure 1) for patient 1.

FIGURE 3. A, Bet v 1-specific IgE levels (y-axis: kUA/L), B, Bet v 1-specific basophil activation (y-axis: percentages of activated basophils) in response to three different concentrations of Bet v 1 (x-axis) C, Mal d 1-specific IgE levels (y-axis: kUA/L), D, Mal d 1-specific basophil activation (y-axis: percentages of activated basophils) in response to three different concentrations of Mal d 1 (x-axis), and E, skin sensitivity to buffer (negative control), histamine (positive control) and apple determined at three different time points (Figure 1) for patient 2.